## SEQUENCE LISTING

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   KURAMITSU, SEIKI
   KAGAMIYAMA, HIROYUKI
- <120> MUTANT KANAMYCIN NUCLEOTIDYLTRANSFERASE AND A METHOD OF SCREENING THERMOPHILIC BACTERIA USING THE SAME
- <130> 04853.0048-00000
- <140> 09/697,186
- <141> 2000-10-27
- <150> JP 309616/1999
- <151> 1999-10-29 /
- <160> 20
- <170> PatentIn Ver. 2.1
- <210> 1
- <211> 253
- <212> PRT
- <213> Artificial Sequence
- <220>
- <223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression
- <400> 1
- Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15
- His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
  20 25 30
- Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
  35 40 45
- Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe 50 55 60
- Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Tyr 65 70 75 80
- Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp
  85 90 95
- Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser 100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 2

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 2

Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe 50 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu 195 200 205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 3

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 3

Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe
50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

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										4										
1	Ser (	Glu (	Glu	Ile	Leu 85	Leu	Asp	Tyr	Ala	Ser 90		Val	Glu	Pro	Asp 95	Trp				
•	Pro I	Leu '	Thr	His 100	Gly	Arg	Phe	Phe	Ser 105	Ile	Leu	Pro	Ile }	Tyr 110	Asp	Pro				
1	Gly		Tyr 115	Phe	Glu	Lys	Val	Tyr 120	Gln	Thr	Ala	Lys	Ser 125	Val	Glu	Ala				
	Gln I	.ys 1	Phe	His	Asp	Ala	Ile 135	Сув	Ala	Leu	Ile	Val 140	Glu	Glu	Leu	Phe				
	Glu T 145	yr A	Ala	Gly	Lys	Trp 150	Arg	Asn	Ile	Arg	Val 155	Gln	Gly	Pro	Thr	Thr 160				
1	Phe L	eu E	Pro	Ser	Leu 165	Thr	Val	Gln	Val	Ala 170	Met	Ala	Gly	Ala	Met 175	Leu			,	
	Ile G	ly I		His 180	Hiș	Arg	Ile	Cys	Tyr 185	Thr	Thr	Ser	Ala	Ser 190	Val	Leu	·	4		•
	Thr G		la ' .95	Val	Lys	Gln		Asp 200	Leu	Pro	Pro	Gly	Tyr 205	Val	Gln	Leu			-	
	Cys G 2	ln L 10	eu '	Val	Met		Gly 215	Gln	Leu	Ser	Asp	Pro 220	Glu	Lys	Leu	Leu				·
	Glu S 225	er L	eu (	Glu .		Phe 230	Trp	Asn	Gly		Gln 235	Glu	Trp	Ala		Arg 240				
Y	His G	ly T	yr 1		Val 2	Asp	Val	Ser		Arg 250	Ile	Pro	Phe							
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,	<223>				of cati		fic	ial	Seque	ence	: Pr	imer	for			•				
	<400> gactgt		g gt	acco	gttg	g acc	gegg	gata	tggt	a				-			35			
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	<211> <212> <213>	35 DNA	fic	ial	Sequ	ence	<b>!</b>													
	<220> <223>				of cati		fici	al s	Seque	nce:	: Pri	imer	for							
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<210> 6
 <211> 35
 <212> DNA
 <213> Artificial Sequence
 <223> Description of Artificial Sequence: Primer for
       PCR amplification
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                                                                        35
 <210> 7
 <211> 35
<212> DNA
<213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Primer for
       subcloning of WT
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                                                                       35
<210> 8
<211> 35
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Primer for
      subcloning of KT3-11 and HTK
<400> 8
gactgtacgc atatgaaagg accaataata atgac
                                                                       35
<210> 9
<211> 35
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Primer for subcloning
gactgtacgc tcgagcgtaa ccaacatgat taaca
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<210> 10
<211> 759
<212> DNA
<213> Staphylococcus aureus
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<220>
<221> CDS
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<400> 10
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I	at	gaa Glu	a at u Il	e Ly	g ga s Gl 0	a cga u Arg	a ata g Ile	a ttg e Lei	g gat 1 Asp 25	Lys	tat Tyr	ggg Gly	gat Asp	gat Asp 30	val	aag Lys	96
Į	gct Ala	att Ile	gg G1 3	y Va	t ta l Ty:	t ggd r Gly	c tct / Sei	ctt Leu 40	ı Gly	cgt Arg	cag Gln	act Thr	gat Asp 45	Gly	Pro	tat Tyr	144
			Il			g atg Met		. Val					Glu			ttc' Phe	192
S						a acc Thr 70	Gly					Glu					240
a S	gc er	gaa Glu	gag Glu	g att	cta Leu 85	cta Leu	gat Asp	tat Tyr	gca Ala	tct Ser 90	cag Gln	gtg Val	gaa Glu	tca Ser	gat Asp 95	tgg Trp	288
P	cg ro	ctt Leu	aca Thr	His 100	Gly	caa Gln	ttt Phe	ttc Phe	tct Ser 105	att Ile	ttg Leu	ccg Pro	att Ile	tat Tyr 110	gat Asp	tca Ser	336
G.	gt ly	gga Gly	tac Tyr 115	Leu	.gag .Glu	aaa Lys	gtg Val	tat Tyr 120	caa Gln	act Thr	gct Ala	aaa Lys	tcg Ser 125	gta Val	gaa Glu	gcc Ala	384
G)	aa ln	acg Thr 130	ttc Phe	cac His	gat Asp	gcg Ala	att Ile 135	tgt Cys	gcc Ala	ctt Leu	atc Ile	gta Val 140	gaa Glu	gag Glu	ctg Leu	ttt Phe	432
ga G1 14	u	tat Tyr	gca Ala	ggc Gly	aaa Lys	tgg Trp 150	cgt Arg	aat Asn	att Ile	cgt Arg	gtg Val 155	caa Gln	gga Gly	ccg Pro	aca Thr	aca Thr 160	480
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at Il	t e	ggt Gly	ctg Leu	cat His 180	cat His	cgc Arg	atc Ile	Cys	tat Tyr 185	acg Thr	acg Thr	agc Ser	gct Ala	tcg Ser 190	gtc Val	tta Leu	576
ac Th	t (	Glu	gca Ala 195	gtt Val	aag Lys	caa Gln	Ser	gat Asp 200	ctt Leu	cct Pro	tca Ser	ggt Gly	tat Tyr 205	gac Asp	cat His	ctg Leu	624

672

720

759

tgc cag ttc gta atg tct ggt caa ctt tcc gac tct gag aaa ctt ctg Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 215 gaa tog ota gag aat tto tgg aat ggg att cag gag tgg aca gaa cga Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg 230 235 cac gga tat ata gtg gat gtg tca aaa cgc ata cca ttt His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe <210> 11 <211> 253 <212> PRT <213> Staphylococcus aureus <400> 11 Met Asn Gly Pro'lle lle Met Thr Arg Glu Glu Arg Met Lys Ile Val His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe 55 60 Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser 105 Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 185 Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu 195

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 12

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 12

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15

Tyr Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr 35 40 / 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe
50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 190 Thr Glu Ala Leu Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220 |

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 13

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 13

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asn Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr 35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe 50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Pro Asp Trp 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser

Gly Gly Tyr Leu Gly Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Leu Val Leu 180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu 195 200 205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 14

<211> 253

<212> PRT

<213> Artificial Séquence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 14

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  $1 \hspace{1cm} 5 \hspace{1cm} 10_{\hspace{1cm}/} \hspace{1cm} 15$ 

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 i 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asn Val Ser Lys Arg Ile Pro Phe 245 250

<210> 15

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 15

Met Ser Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Thr Val Leu 180 185 190

Thr Glu Ala Val Lys Leu Ser Asp Leu Pro Ser Gly Tyr Asp His Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Thr Glu Arg 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 16

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 16

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Thr Glu Phe 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp 85 90 95

Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Thr 100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Leu Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Gly Ala Ser Val Leu 180 185 190

Thr Glu Ala Val Arg Gln Pro Asp Leu Pro Pro Gly Tyr Asp His Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Ala Glu Arg 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 17

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant/enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 17

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro 100 105 110 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 18

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 18

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

1 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe 50 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp 85 90 95 Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250

<210> 19

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 19

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp
85 90 95

Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Pro Glu Lys Leu Leu 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe

<210> 20

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 20

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Glu Ala Glu Phe 50 60

- Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr 65 70 75 80
- Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp 85 90 95
- Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
  100 105 110
- Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala 115 120 125
- Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe 130 135 140
- Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr 145 150 155 160
- Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu 165' 170 175
- Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu 180 185 190
- Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu 195 200 205
- Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu 210 215 220
- Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg 225 230 235 240
- His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe 245 250